

Karyotype Differentiation in Newly Discovered Members of the *Drosophila obscura* Species Group from Yunnan, China

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Abstract: We examined mitotic chromosomes of three newly found members of the *Drosophila obscura* species group, *D. luguensis*, *D. dianensis* and *D. limingi* from Yunnan and compared their karyotypes to those of respective related species. *D. luguensis* possessed a diploid number of 12 chromosomes, comprising 3 pairs of metacentrics (V-shaped), 2 pairs of acrocentrics (rod-like) and 1 pair of micro-chromosomes (dot-like). Both the X and Y were metacentrics. *D. dianensis* and *D. limingi* possessed a diploid number of 10 chromosomes, comprising 1 pair of large V-shaped, 1 pair of moderate-sized V-shaped, 2 pairs of submetacentric (J-shaped) chromosomes, and 1 pairs of dot-like chromosomes, with their X chromosomes were J-shaped and Y chromosomes were short rod-like ones. Based upon the results of chromosomal comparisons, geographic information of the *sinobscura* subgroup, as well inter-specific phylogenetic relationship deduced elsewhere, *D. luguensis* was considered to retain an ancestral form of the karyotype within this subgroup. The present observations have also implied that *D. sinobscura* ($2n = 12$: 2V, 1J, 2R, 1D) might have derived from a sub-lineage of a pre-“*sinobscura-hubeiensis*” lineage by pericentric inversion of moderate-sized metacentric autosomes and *D. hubeiensis* ($2n = 10$: 4V, 1D) from another sub-lineage of the same lineage by centric fusion of 2 pairs of acrocentric autosomes. Similar chromosomal changes of centric fusion, centric fission and/or pericentric inversion might have occurred during the course of species divergences between *D. dianensis* and *D. subsilvestris* ($2n = 12$: 3V, 2R, 1D), an European member closely related to *D. dianensis*, and between *D. limingi* and *D. tsukubaensis* ($2n = 12$: 3V, 2R, 1D), an East Asian member near to *D. limingi*.

Key words: *Drosophila obscura* species group; Karyotype differentiation; China

中国云南果蝇属暗果蝇种组的核型分化

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摘要: 观察了新近发现于我国云南的果蝇属暗果蝇种组 (*Drosophila obscura* species group) 种类 *D. luguensis*、*D. dianensis* 和 *D. limingi* 的有丝分裂中期核型, 并将 3 个种的核型与各自的近缘种类进行了比较。*D. luguensis* 具 $2n = 12$ 条染色体, 包括 3 对中央着丝粒 (V 形) 染色体、2 对近端着丝粒 (棒状) 染色体以及 1 对微小 (点状) 染色体。其中 X 和 Y 染色体均为中央着丝粒染色体。*D. dianensis* 和 *D. limingi* 具 $2n = 10$ 条染色体, 包括 1 对大的 V 形常染色体, 1 对小的 V 形常染色体, 2 对 J 形 (亚中着丝粒型) 常染色体和 1 对点状染色体。其中 X 染色体为 J 形, Y 染色体为短棒状。基于核型比较的结果以及 *D. sinobscura* 亚组地理分布的资料, 结合种间系统发育关系研究结果, 认为 *D. luguensis* 可能保留了该亚组祖先种类的核型。*D. sinobscura* 的核型

Received date: 2004-01-18; Accepted date: 2004-03-29

Foundation items: The present study was supported by the National Natural Science Foundation of China (30021004), Chinese Academy of Sciences (KSCX2-1-05) and Japan Society for the Promotion of Science (No. 12375002)

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收稿日期: 2004-01-18; 接受日期: 2004-03-29

($2n = 12$: 2V, 1J, 2R, 1D) 可能由一个 pre- "*sinobscura-hubeiensis*" 谱系的一个分支通过臂间倒位演化而来，而 *D. hubeiensis* 的核型 ($2n = 10$: 4V, 1D) 可能由该谱系的另一分支通过着丝粒融合 (2 对近端着丝粒常染色体的融合) 而形成。推测在 *D. dianensis* 和近缘欧洲种 *D. subsilvestris* ($2n = 12$: 3V, 2R, 1D) 间、*D. limingi* 和东亚近缘种 *D. tsukubaensis* ($2n = 12$: 3V, 2R, 1D) 间的物种分化过程中，可能有相似的染色体变异类型发生。

关键词：暗果蝇种组；核型分化；中国

中图分类号：Q969.462.1; Q343.2 **文献标识码：**A **文章编号：**0254-5853(2004)03-0236-06

Chromosome configuration has provided us with invaluable information when we consider the phylogeny and evolution of *Drosophila* (for the reviews, see Patterson & Stone, 1952; Throckmorton, 1982; Levitan, 1982).

The *Drosophila obscura* species group has been well studied from an evolutionary point of view by North American and European researchers (Dobzhansky & Epling, 1944; Buzzati-Traverso & Scossiroli, 1955; Powell et al, 1976). Lakovaara & Saura (1982) and Powell (1997) had reviewed phylogenetic relationships within this group. China has been nearly a virgin territory for the *obscura* group until recently, irrespective of its importance in geographical distributions. During recent years, five new members of this group have been found from China: *D. sinobscura* Watabe, 1996 from Taiwan, *D. hubeiensis* Sperlich et Watabe, 1997 from Hubei, and subsequently *D. dianensis* Gao et Watabe, 2003, *D. limingi* Gao et Watabe, 2003 and *D. luguensis* Gao et Toda, 2003 from Yunnan. Further, new information on geographic distributions of the *obscura* group has been accumulated in the mainland of China (Gao et al, 2003).

In the present study, metaphase chromosomal configurations of newly described species of the *obscura* group were analyzed and compared to those of their closely related ones. Based upon results of karyotype comparison and geographic information, an evolutionary process was proposed within the *sinobscura* species subgroup, which was recently established by three Chinese species (Gao et al, 2003), and a hypothetical model of chromosome differentiations within several Old World lineages of the *obscura* group was discussed.

1 Materials and Methods

1.1 Iso-female strains

Iso-female strains of seven species were used for chromosomal studies: *D. luguensis* collected from Lugu Lake Nature Reserve, Ninglang, Yunnan; *D. sinobscura* collected from Chitou, Taiwan; *D. hubeiensis* collected from Gaoligongshan Nature Reserve, Lushui, Yunnan and from Shennongjia, Hubei; *D. dianensis*, *D. limingi* and *D. tsukubaensis* Takamori et Okada, 1983 from Jiaoye Park, Kunming, Yunnan; *D. subsilvestris* Hardy et Kaneshiro, 1968 from Tübingen, Germany. Although chromosomes of *D. sinobscura*, *D. hubeiensis* (Hubei strain), *D. subsilvestris* and *D. tsukubaensis* (Japanese strain) had already been studied, they were re-examined for the detailed comparison of karyotypes and for checking intra-specific geographic variations.

1.2 Preparations of mitotic metaphase chromosomes

Preparations of chromosomes were made by the method of Imai et al (1977). Neuroblasts of the 3rd instar larvae were dissected in Ringer's solution and fixed with Carnoy solution. They were treated with 0.1 mg/ml of colchicines solution, stained with 4% Giemsa solution, and then air-dried. About 100 metaphase plates were observed in each species.

2 Results and Analyses

Male metaphase configurations of seven species in the *obscura* species group were shown in Fig.1. *Drosophila luguensis* possessed a diploid number of 12 chromosomes, comprising 2 pairs of moderate-sized metacentric (V-shaped) autosomes, 2 pairs of acrocentric (rod-like) autosomes, a pair of micro-chromosomes (dot-like), and a pair of sex chromosomes, with both X and Y were metacentric (Fig.1A). The karyotype of *D. sinobscura* closely resembled that of

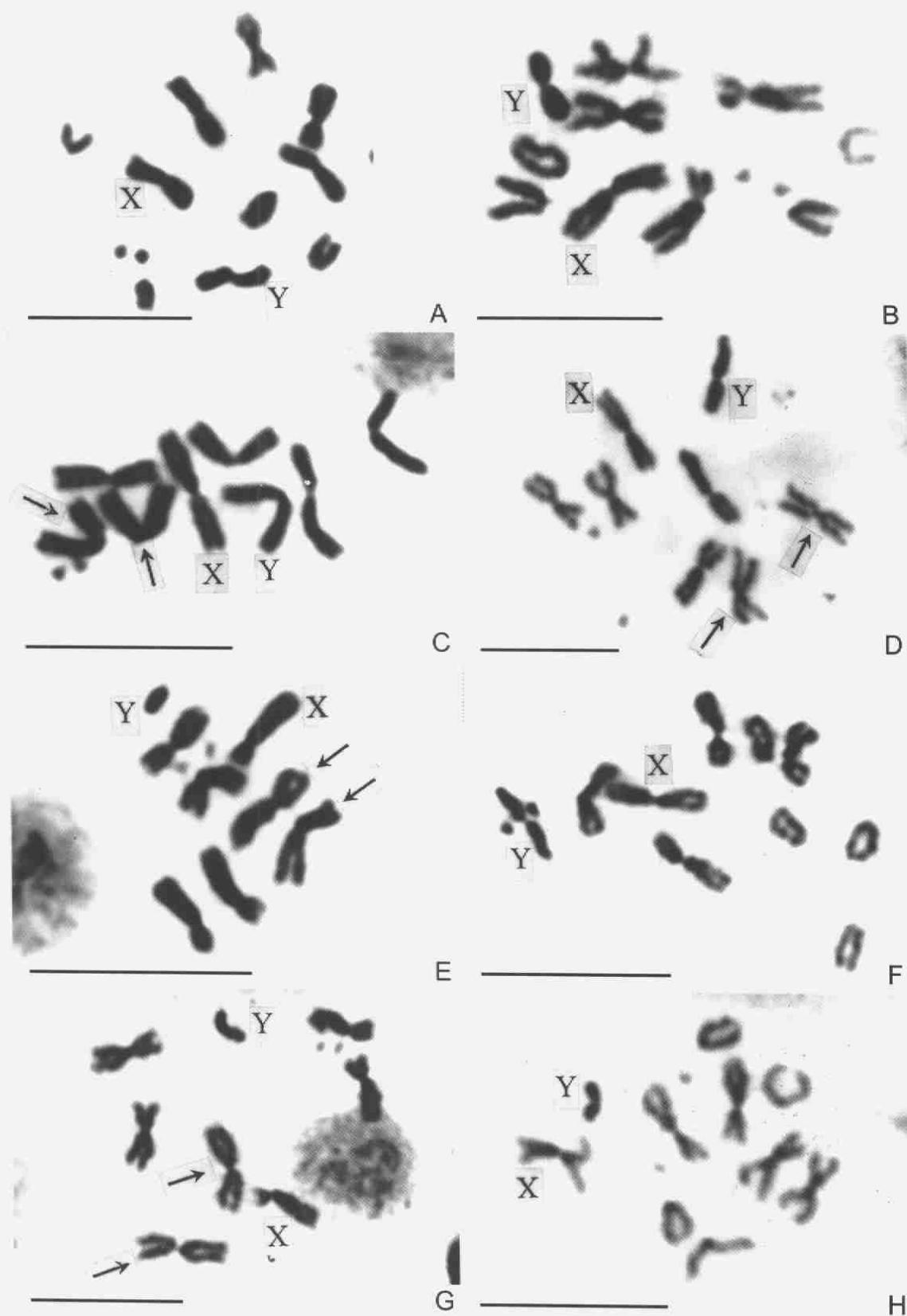


Fig. 1 Male metaphase chromosomes of seven species of the *Drosophila obscura* species group
 A: *D. luguensis*; B: *D. sinoboscra*; C: *D. hubeiensis* (Yunnan strain); D: *D. hubeiensis* (Hubei strain); E: *D. dianensis*; F: *D. subsilvestris*; G: *D. limingi*; H: *D. tsukubaensis* (Yunnan strain). Arrows indicate large metacentric autosomes. Bars represent 10 μm .

D. luguensis, except for that the former had a pair of moderate-sized submetacentric (J-shaped) autosomes being absent in the latter (Fig. 1B). Ganglion cells of the Yunnan strain of *D. hubeiensis* showed a diploid number of 10 chromosomes, consisting of 4 pairs of V-shaped and a pair of dot-like chromosomes (Fig. 1C – D), with one pair of the metacentric autosomes was larger in size than the other autosomes. Mitotic preparations of *D. dianensis* showed $2n = 10$ chromosomes, consisting of 2 pairs of metacentrics, 2 pairs of submetacentrics and a pair of micro-chromosomes. Its X was submetacentric and Y acrocentric (Fig. 1E). On the other hand, *D. subsilvestris*, a European member closely related to *D. dianensis* (Gao et al, 2003) showed $2n = 12$ chromosomes (3V, 2R, 1D) with metacentric X and Y (Fig. 1F). One pair of relative large metacentric autosomes was remarkable in *D. dianensis*. Neuroblast cells of *D. limingi* showed $2n = 10$ chromosomes, comprising 2 pairs of metacentrics, 2 pairs of submetacentrics and 1 pair of micro-chromosomes. Its X was J-shaped and Y was V-shaped (Fig. 1G). *D. tsukubaensis*, an East Asian member somewhat near to *D. limingi*, showed $2n = 12$ chromosomes (3V, 2R, 1D) with metacentric X and Y (Fig. 1H).

Our preparations present a selfsame chromosome configuration for the Yunnan strain of *D. hubeiensis* as that of the Hubei strain of same species (Watabe & Sperlich, 1997). The present result of *D. subsilvestris* is identical to that studied by Knight (1956) except for submetacentric Y; karyotype of the Kunming strain of *D. tsukubaensis* is identical to that of the Japanese strain of same species (Takamori & Okada, 1983).

3 Discussion

The three species, *D. luguensis*, *D. sinobscura* and *D. hubeiensis* of the *sinobscura* subgroup are quite similar to each other in external morphology and genitalia structure. In particular, the latter two species are morphologically undistinguishable sibling species (Watabe & Sperlich, 1997; Gao et al, 2003). Such a relationship obtained from the morphological comparison is supported by the molecular data of DNA se-

quences of the mitochondrial *COI*, *COII* and *ND2* genes and of the nuclear *Adh* gene. In addition, affinities between *D. dianensis* and *D. subsilvestris*, as well between *D. limingi* and *D. tsukubaensis* are affirmed by the same data set with high bootstrap value support (Gao et al, unpublished data). *D. luguensis* and *D. sinobscura* possess $2n = 12$ chromosomes, lacking any large metacentric autosomes but having 2 pairs of acrocentric autosomes, whereas *D. hubeiensis* shows $2n = 10$, with large metacentric autosomes and without any acrocentric ones. The differences in chromosome number between $2n = 12$ and $2n = 10$ can be explained by centric fusion of 2 pairs of acrocentric autosomes found both in *D. luguensis* and *D. sinobscura* or by centric fission of 1 pair of large metacentric autosomes found in *D. hubeiensis*. Reduction of chromosome number is more likely to have occurred in the family Drosophilidae than increase, since the formation of 2 pairs of acrocentric chromosomes from a pair of metacentric ones requires the addition of centromeres (Patterson et al, 1940; Patterson & Stone, 1952; Throckmorton, 1982; Clayton & Guest, 1986; Watabe & Sperlich, 1997). Thus, the former hypothesis of “centric fusion” appears more probable than the latter one (Fig. 2).

Compared to *D. hubeiensis* (4V, 1D), *D. luguensis* (3V, 2R, 1D) and *D. sinobscura* (2V, 1J, 2R, 1D) are considered to be chromosomally nearer to the hypothetical most recent common ancestor of the *sinobscura* subgroup, which might presumably possess $2n = 12$ (3V, 2R, 1D) chromosomes, since a diploid number of 12 is considered to be more primitive than 10 within the *obscura* species group.

Drosophila hubeiensis is widely distributed in middle and southern parts of Mainland China. *D. luguensis* occurs abundantly at Lijiang district, in Yun-Gui Plateau, with an elevation of about 2 800 m, and it is sympatric to *D. hubeiensis* there (Gao et al, 2003). *D. sinobscura* is presently known from Taiwan Island, alone. The present geographic and chromosomal evidences imply that the *sinobscura* subgroup might have emerged in Mainland China, probably its southern districts (Gao et al, 2003). Thereafter, an

| | X | Autosomes | Y |
|------------------------|---|-----------|---|
| <i>D.luguensis</i> | X | X X n n . | X |
| <i>D.sinobscura</i> | X | X X n n . | X |
| <i>D.hubeiensis</i> | X | X X X . | X |
| <i>D.dianensis</i> | X | X X X . | n |
| <i>D.subsilvestris</i> | X | X X n n . | X |
| <i>D.limigi</i> | X | X X X . | n |
| <i>D.tsukubaensis</i> | X | X X n n . | X |

Fig.2 Schematic representation of chromosomes in the *Drosophila obscura* species group

ancestor of the *sinobscura* subgroup might have diverged into two lineages, a pre-“*luguensis*” lineage giving rise to *D.luguensis* and a pre-“*sinobscura-hubeiensis*” lineage, which subsequently diverged into two sub-lineages, a pre-“*sinobscura*” and a pre-“*hubeiensis*” sub-lineages. The pre-“*luguensis*” lineage probably retained the ancestral form of chromosomal configurations ($2n = 12$: 3V, 2R, 1D). Chromosomes of *D.sinobscura* ($2n = 12$: 2V, 1J, 2R, 1D) might have derived from the pre-“*sinobscura*” sub-lineage by pericentric inversions of moderate-sized metacentric autosomes, whereas those of *D.hubeiensis* (4V, 1D) from the pre-“*hubeiensis*” sub-lineage by centric fusion of 2 pairs of acrocentrics (Fig.3).

The haploid chromosome number of 6 has been considered to be “basic” status in the genus *Drosophila*

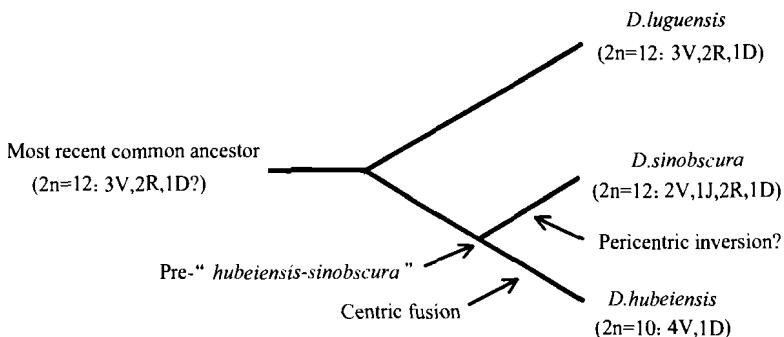


Fig.3 A hypothetical scenario of chromosome evolution within the *Drosophila sinobscura* species subgroup

since reviewed by Muller (1940) and Patterson & Stone (1952). Clayton & Guest (1986) stated that 100 out of the 141 species analyzed had a haploid number of $n = 3$ or $n = 4$, and that only 4 species, *D.obscura*, *D.subobscura*, *D.imaiii* and *D.subsilvestris* retain the basic number of $n = 6$, all of which belong to the *obscura* group. Thereafter, three species of the *obscura* group, *D.tsukubaensis*, *D.sinobscura* and *D.luguensis*, have added to the group with the basic haploid chromosome number of 6. If the chromosome number of $n = 6$ would be more primitive than that of $n = 5$ in a sense of chromosomal evolution, *D.dianensis* and *D.limigi* might be more likely derived species compared to the respective relatives, *D.subsilvestris* and *D.tsukubaensis*. And karyotype

comparison between *D.dianensis* and *D.subsilvestris*, as well between *D.limigi* and *D.tsukubaensis* indicate similar karyotype changes in these species pairs to that in the *sinobscra* species subgroup. That is, centric fusion or fission and/or pericentric inversion might have occurred between *D.dianensis* and *D.subsilvestris* and between *D.limigi* and *D.tsukubaensis*.

A few examples of pericentric inversion have been observed in the *obscura* group (Miller & Stone, 1962; Brehm & Krimbas, 1991; Segarra & Aguadé, 1992). One pair of submetacentric autosomes found in *D.dianensis* and *D.limigi* might have resulted from pericentric inversion of metacentric autosomes found in *D.subsilvestris* and *D.tsukubaensis*, respectively, although a further study on banding patterns of salivary

gland chromosomes is desirable to analyze the pericentric inversion. More steps of karyotypic changes are needed for tracing chromosomal evolution between *D. dianensis* and *D. subsilvestris* and between *D. limingi* and *D. tsukubaensis*. This suggests a possibility of the presently undiscovered members related to those species in Mainland China.

Acknowledgements: The authors wish to express

their cordial thanks to Prof. TODA MJ (Hokkaido University), Dr. HARING E (Museum of Natural History, Vienna), Dr. BÄCHLI G (Universität Zurich-Irchel), professor LIU AH, Mrs. LIN SY and Mrs. WANG RF (Kunming Institute of Zoology, the Chinese Academy of Sciences) for their invaluable advices, and to Prof. SPERLICH D (University of Tübingen) and Dr. TAKAMORI H (Tokyo Gakugei University) for providing us with living strains.

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